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Marek's Disease Virus Late Protein Expression in Feather Follicle Epithelial Cells as Early as 8 Days Postinfection

Keith W. Jarosinski
Contact Address: keith-jarosinski@uiowa.edu

Important Findings

Marek's disease virus (MDV) or Gallid herpesvirus 2 (GaHV-2) is a lymphotropic alphaherpesvirus that Marek's disease. Former studies have demonstrated that MDV is spread from chicken to chicken about 2 wk postexposure in infectious dander shed from infected chickens. More recent reports, using highly sensitive quantitative polymerase chain reaction (PCR) analyses of dander from infected chickens, suggested MDV replicates and is shed from the chicken much earlier (5–7 days). However, detection of viral DNA in chicken dander does not indicate whether fully infectious virus is present. To determine if viral replication is present in the skin of infected chickens at these early times, expression of a late viral protein indicative of fully productive virus replication was evaluated using fluorescent microscopy. Though viral DNA can be detected as early as 6 days postinfection (p.i.), late viral protein expression, indicative of infectious virus production, occurs 2–3 days after DNA detection, but earlier than previously thought.

Significance of Findings

The data presented here confirm that MDV DNA is present in feather follicles as early as 6 days p.i. and conclusively show that viral protein expression is evident at 8 days p.i. during infection with virulent MDV. Additionally, during infection with attenuated MDV, although viral DNA can also be detected as early as 6 days p.i., viral protein expression is delayed 1–2 days relative to infection with virulent recombinant MDV (rMDV).

Additional Information

Marek's disease (MD) is caused by MDV or GaHV-2 in chickens. Clinical symptoms of MD include depression, neurological signs such as paralysis and ataxia, and the development of lymphoproliferative disease characterized by solid tumors in the viscera and other organs. Natural infection is believed to begin through inhalation of virus, after which primary cytolytic replication in B and then T lymphocytes ensue. Following lytic infection, latency is established in activated $CD4^+$ T cells that can be transformed into highly proliferative T cell lymphomas. Irrespective of the transformation event, infected lymphocytes migrating in the skin transfer virus to feather follicle epithelial (FFE) cells that leads to the production of infectious particles that are shed into the environment, providing a continuous source of infectious virus.

Much attention has been placed on the oncogenic aspect of MD and vaccinology since the identification of MDV as the etiologic agent. After the identification of the mode of transmission of MDV, that being infectious dander shed from infected chickens approximately 14 days p.i., information pertaining to this important aspect of the virus life cycle has been limited until recently. The identification of MDV encoded proteins that are essential for transmission has shed some light onto this area of research. Seminal studies in the late 1960s and early 1970s elucidated important aspects of MDV transmission. These reports suggested that infectious virus was present as early as 7 days p.i. in both nasal washings and feces; however, the data at that time were conflicting. Subsequently it was determined the main source of transmission for MDV was dust and dander shed from infected chickens. Since then, it has been accepted that MDV reaches the FFE and produces infectious virus around 10–12 days p.i., followed by shedding of infectious virus into the environment at an infective dose around 14 days p.i.. However, more recent studies using highly sensitive PCR to detect viral genomes found that virus was detectable as early as 7 days p.i. in feather tips and as early as 5–7 days p.i. in dander shed from the chicken. The early detection of MDV DNA in these studies suggested transmission of MDV may occur earlier than originally thought, especially when more virulent viruses present today have earlier and more pronounced replicative rates.

Advances in molecular cloning and generation of rMDV allow us to revisit some of the long-standing questions that could not be addressed during the early studies on the elucidation of MDV pathogenesis. For example, fusing the enhanced green fluorescent protein (eGFP) to the C-terminus of the late UL47 (VP13/14) tegument protein of MDV (UL47-eGFP) led to the discovery that this protein is expressed at very low levels during *in vitro* MDV replication, while it is expressed abundantly in FFE cells in the skin. This finding circumstantially linked late protein expression levels to the production of infectious virus in FFE cells *in vivo*, and lack thereof, in the case of the strictly cell-associated nature of MDV during *in vitro* replication. Importantly, this study also showed that fusing the fluorescent protein to the viral UL47 protein resulted in no loss of pathogenicity, showing this is a powerful tool for studying MDV replication in the FFE cells of infected chickens. This approach was again utilized recently to generate additional fluorescent viruses using the monomeric red fluorescent protein (mRFP) fused to the UL47 protein (UL47-mRFP) in virulent and attenuated rMDV to examine dual infection of FFE cells with two viruses. These viruses were employed in the present study to address two important questions. First, when is the earliest time point at which expression of late MDV protein is detected in FFE cells, which would be indicative of a fully productive replicative cycle? Second, is there a difference between virulent and attenuated viruses with respect to when late protein expression can be detected and the relative number of infected follicles? Using qPCR for viral DNA and microscopy for viral antigens, the earliest time point at which viral DNA could be detected was at 6 days p.i. for both virulent and attenuated rMDV, consistent with earlier results, while expression of the late MDV protein, UL47-mRFP, could be detected at 8 days p.i during infection with virulent rMDV. Detection of late protein expression was delayed for attenuated viruses by 1–2 days.

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